Accuracy of Xpert® MTB/RIF in diagnosing extrapulmonary tuberculosis in Indian children

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ABSTRACT

Background. Diagnosing extrapulmonary tuberculosis (EPTB) can be challenging because of a variety of presentations. We assessed the accuracy of the Xpert MTB/RIF assay in diagnosing EPTB in children.

Methods. Of the 255 children diagnosed to have tuberculosis (TB) who underwent testing by the Xpert MTB/RIF assay at the TB clinic from December 2014 to April 2017, 182 had EPTB and were included in the study. The diagnostic accuracy, specificity and sensitivity of the Xpert assay were calculated with *Mycobacterium* growth indicator tube (MGIT) as a reference standard.

Results. Lymph node TB was present in 58 (32%) children, 37 (20%) had neurological TB, 36 (20%) had bone TB, 31 (17%) had pleural TB, 15 (8%) had abdominal TB, 2 (1%) had abscess, 2 (1%) had congenital TB and disseminated TB was seen in 1 (0.4%) child. Xpert MTB/RIF assay was positive in 84 (46.2%) patients. The sensitivity and specificity of the Xpert MTB/RIF assay were 72% and 72.04%, respectively. Compared to MGIT, a kappa coefficient of 0.44 shows moderate agreement between the Xpert assay and MGIT. The sensitivity of Xpert MTB/RIF assay in abdominal TB, bone TB, lymph node TB, neurological TB and pleural TB was 50% (15%–85%), 72.7% (15.9%–86.9%), 80.8% (62.1%–91.5%), 75% (50.5%–90%) and 25% (4.6%–70%), respectively. The specificity of abdominal TB, bone TB, lymph node TB, neurological TB and pleural TB was 83.3% (43.7%–97%), 69.2% (42.4%–87.3%), 55.2% (37.6%–71.6%), 85% (64%–94.8%) and 82.6% (62.9%–93%), respectively. Forty-seven (26%) patients had drug-resistant TB (DR-TB), of which 15 (8%) were rifampicin-resistant (RR), 2 (1%) were polyresistant, 14 (8%) had multi-DR (MDR), 15 (8%) had pre-extremely DR (XDR) and 1 (1%) had XDR-TB. Of the 15 patients with MDR-TB, Xpert MTB/RIF assay detected only 10 (71%) as RR ($p=0.06$). Of the 15 pre-XDR cases, Xpert MTB/RIF detected only 8 (53%) as RR ($p=0.02$).

Conclusion. Xpert MTB/RIF assay is useful in the diagnosis of EPTB. It shows good concordance with MGIT. However, it may be negative in patients with DR-TB.

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INTRODUCTION

India has the largest burden of tuberculosis (TB) cases in the world. The WHO TB statistics for 2015 gave an estimated incidence of 2.2 million cases of TB in India out of a global incidence of 9.6 million and about 500 000 annual deaths due to the disease.[1,2]

Traditionally, a diagnosis of multidrug-resistant TB (MDR-TB) infection requires mycobacterial culture and phenotypic drug-susceptibility testing (DST). This approach requires relatively advanced laboratory capacity and intensive labour and takes 1–3 months for the results. The treatment for MDR-TB is complex, lengthy and expensive, and diagnostic delays with MDR-TB are associated with worse clinical outcomes and increased rates of transmission.[3]

In 2011, the WHO recommended the use of rapid molecular genotyping methods such as Xpert MTB/RIF assay, which is based on nucleic acid amplification technique (NAAT), over conventional phenotypic methods for drug sensitivity testing for diagnosis.[4] The Xpert is becoming a principal screening tool for diagnosing rifampin-resistant *Mycobacterium tuberculosis* complex.

Worldwide, extrapulmonary TB (EPTB) accounts for almost a quarter of all TB cases and even a higher percentage in HIV-infected individuals and children. Existing tests for the diagnosis of EPTB are limited in accuracy and time-consuming and often require invasive procedures and special expertise.[5] Culture of pleural fluid, lymph node aspirate and cerebrospinal fluid (CSF) has low sensitivity, although repeat examination of the CSF or the use of NAATs may increase sensitivity and is associated with high specificity (>98%).[6] Culture specificity is 100% if the presence of *M. tuberculosis* (MTB) complex is confirmed. Often, biopsy with culture and histopathological examination is necessary to achieve a diagnosis. An alternative to this method is the Xpert assay. The Xpert has high accuracy for detection of pulmonary TB (PTB; sensitivity 89% and specificity 99%), but its accuracy for EPTB detection is not clear.[5] We did a retrospective study to assess the accuracy of Xpert MTB/RIF assay in diagnosing EPTB in children.

METHODS

We did a single-centre retrospective study at our paediatric TB clinic in a tertiary referral children's hospital. The study did not involve any clinical interaction with the participants or administration of any therapeutic agents. The study participants included patients diagnosed with EPTB either clinically or bacteriologically from December 2014 to April 2017. All children investigated with the Xpert MTB/RIF assay for the diagnosis of TB in that period were included in the study.

A total of 255 children diagnosed to have TB underwent testing by the Xpert MTB/RIF assay. Of the 255, 182 had EPTB and were included in the study. The remaining 73 children were excluded, of which 70 were diagnosed with PTB and 3 had insufficient medical records. Types of EPTB comprised pleural TB, abdominal TB, lymph node TB, bone TB, neurological TB, congenital TB and disseminated TB.

Details of the 182 children were noted from the patient records, and their demographic data, investigations done at the time of diagnosis, sample used for investigations, type of TB and presence of resistant TB were recorded.

Investigations noted from the records included the Xpert MTB/RIF assay, TB *Mycobacterium* growth indicator tube (TB MGIT) and acid-fast bacilli (AFB) smear microscopy. Specimens taken for investigations were taken from normally sterile sites such as pleural fluid, CSF, ascitic fluid, tissue biopsy, lymph node biopsy, gastric lavage, bronchoalveolar lavage (BAL), sputum, and pus, if present at the site of infection. If multiple specimens showed positive results from the same patient, the result of only one specimen was included in the study. Therefore, per participant only one specimen was included for analysis in this study. No samples were rejected due to inadequacy or inappropriateness.

Specimens were collected in separate sterile containers for MGIT culture and GeneXpert MTB/RIF assay analysis. Aseptic homogenization inside a biosafety cabinet was used to homogenize each sample.

For GeneXpert MTB/RIF assay, the sample collected was treated with a sample reagent (SR) consisting of NaOH and isopropanol as per the manufacturer's instruction. A 2:1 ratio of SR to unprocessed sample was mixed in a tube which was incubated for a period of 15 minutes at room temperature, during which the tubes were manually agitated twice. After the incubation period was complete, 2 ml of the sample was added to liquid buffers and lyophilized reagent beads present in the multichambered plastic cartridge for DNA extraction and hemi-nested real-time polymerase chain reaction (RT-PCR). Once the sample was loaded, the cartridge was inserted in the Xpert machine and the remaining assay steps were automatically completed.

For MGIT, the samples were processed using the N-acetyl-L-cysteine-NaOH method. One millilitre of sterile phosphate-buffered saline (pH 6.8) was used for suspension of the sediment. After processing, MGIT 960 vials were used for inoculation of 0.5 ml of the processed specimen, as described by the manufacturer. Lowenstein–Jensen (LJ) medium slants were inoculated with 0.2 ml of the processed sample. The MGIT 960 vials were then incubated for a maximum duration of 6 weeks or until a specimen was positive. The inoculated LJ medium slants were examined for growth once a day for the first week and once a week for 12 weeks thereafter. Ziehl–Neelsen staining was used to confirm the presence of AFB in all positive MGIT vials, and the PNBA assay was used to identify *M. tuberculosis* complex. Gram-staining of the smear and subculture in blood agar were used if there was obvious turbidity in the MGIT vial. Subculture of 0.2 ml of positive broth on additional LJ medium slants was also done. This slant was used to rule out MTB and non-tuberculous *Mycobacterium*-mixed infections.

Culture growth on media was considered as the standard for the diagnosis of EPTB. In this study, MGIT was used as a reference standard as it employs the formation of culture on liquid media. The diagnostic accuracy, specificity and sensitivity of the Xpert assay were calculated, keeping MGIT results as a reference standard. The accuracy of the investigations depending on the type of EPTB and specimen taken was also calculated. The results of the investigations were compared for correlation using Cohen's kappa correlation factor for each type of TB and specimen as well as overall.

Statistical analysis was done using SPSS 16.0. Baseline characteristics were evaluated using descriptive statistics. The sensitivity, specificity, Cohen's kappa correlation and diagnostic accuracy were evaluated using screening and diagnostic tests with statistical significance at a 95% confidence interval.

RESULTS

The mean (SD) age was 6.5 (4.2) years. The man-to-woman ratio was 2.03:1. Lymph node TB was present in 58 (32%) children, 37 (20%) children had neurological TB, 36 (20%) had bone TB, 31 (17%) had pleural TB, 15 (8%) had abdominal TB, 2 (1%) had abscess, 2 (1%) had congenital TB and disseminated TB was seen in 1 (0.4%) child. The specimens from various anatomical sites included 62 (34%) biopsies, 38 (41%) CSF samples, 29 (16%) pleural fluid samples, 25 (14%) pus samples, 13 (7%) gastric lavage samples, 6 (3%) ascitic fluid samples, 4 (2%) fine-needle aspirations, 1 (1%) BAL, 1 (1%) bone tissue sample, 1 (1%) endotracheal tube sample and 2 (1%) sputum samples. All 182 samples were tested with the Xpert MTB/RIF assay, of which 84 yielded positive (46.2%) results. Only 168 of the 182 participants were tested with TB MGIT culture, of which 75 (44.6%) grew MTB. The results of TB MGIT and Xpert MTB/RIF assay in each specimen and type of EPTB are given in Tables I and II. Fifteen (14%) specimens of 109 tested showed

TABLE I. Efficacy of Xpert MTB/RIF assay and *Mycobacterium* growth indicator tube (MGIT) in different types of extrapulmonary tuberculosis

Type of tuberculosis	Xpert MTB/RIF assay			MGIT culture		
	Positive results (*n*)	Total tested (*n*)	%	Positive results (*n*)	Total tested (*n*)	%
Abdominal	5	15	33.3	4	10	40
Abscess	1	2	50	2	2	100
Bone	21	36	58.3	22	35	62.9
Congenital	1	2	50	0	2	0
Disseminated	1	1	100	1	1	100
Lymph node	34	58	58.6	26	55	47.3
Neurological	16	37	43.2	16	36	44.4
Pleural	5	31	16.1	4	27	14.8
Total	84	182	46.2	75	168	44.6

TABLE II. Efficacy of Xpert MTB/RIF assay and *Mycobacterium* growth indicator tube (MGIT) as per specimen type

Specimen	Xpert MTB/RIF assay			MGIT culture		
	Positive results (*n*)	Total tested (*n*)	%	Positive results (*n*)	Total tested (*n*)	%
Ascitic fluid	1	6	16.7	1	4	25
BAL	0	1	0	0	1	0
Biopsy	39	62	62.9	33	61	54.1
Bone tissue	0	1	0	0	1	0
CSF	16	38	42.1	17	37	45.9
ET aspirate	1	1	100	1	1	100
FNAB	0	4	0	1	4	25
Gastric lavage	8	13	61.5	1	8	12.5
Pleural fluid	4	29	13.8	4	26	15.4
Pus	15	25	60	17	24	70.8
Sputum	0	2	0	0	1	0

BAL bronchoalveolar lavage ET endotracheal tube CSF cerebrospinal fluid FNAB fine-needle aspiration biopsy

TABLE III. Sensitivity, specificity and diagnostic accuracy of the Xpert MTB/RIF assay for different types of extrapulmonary tuberculosis

Type of tuberculosis	Sensitivity (%)	Specificity (%)	Cohen's κ	p value	Diagnostic accuracy (%)
Abdominal	50.0 (15–85)	83.3 (43.7–97)	0.35 (0.25–0.95)	0.5	70.0 (39.68–89.22)
Bone	72.7 (15.9–86.9)	69.2 (42.4–87.3)	0.41 (0.08–0.74)	0.375	71.4 (54.94–83.67)
Lymph node	80.8 (62.1–91.5)	55.2 (37.6–71.6)	0.35 (0.1–0.74)	0.049	67.3 (54.1–78.19)
Neurological	75.0 (50.5–90)	85.0 (64–94.8)	0.60 (0.28–0.93)	0.5	80.6 (64.97–90.25)
Pleural	25.0 (4.6–70)	82.6 (62.9–93)	0.07 (–0.3–0.4)	0.5	74.1 (55.32–86.83)

TABLE IV. Sensitivity, specificity and diagnostic accuracy of the Xpert MTB/RIF assay for different types of specimens

Type of sample	Sensitivity (%)	Specificity (%)	Cohen's κ (%)	p value	Diagnostic accuracy (%)
Biopsy	84.9 (69–93.3)	60.7 (42.41–76.42)	0.46 (0.22–0.7)	0.1	73.8 (61.56–83.16)
Cerebrospinal fluid	70.6 (46.87–86.7)	85.0 (63.9–94.7)	0.56 (0.24–0.88)	0.36	78.4 (62.8–88.61)
Gastric lavage	100.0 (20.7–100)	14.3 (2.57–51.3)	0.04 (–0.15–0.23)	0.02	25.0 (7.15–59.07)
Pleural fluid	25.0 (4.6–70)	86.4 (66.7–95.3)	0.11 (–0.27–0.5)	0.34	76.9 (57.95–88.97)
Pus	64.3 (38.76–83.66)	66.7 (30–90.32)	0.27 (–0.15–0.69)	0.25	62.5 (42.71–78.84)

AFB on smear. The overall results show that the sensitivity of Xpert MTB/RIF is more than the sensitivity of MGIT, the reference standard, however not significantly (p=0.28). Overall, the sensitivity and specificity of the Xpert MTB/RIF assay were 72% (60.9%–80.9%) and 72.04% (62.19%–80.15%), respectively, compared to MGIT; a kappa coefficient of 0.44 (0.29–0.59) shows moderate agreement between the Xpert assay and MGIT. The diagnostic accuracy of Xpert MTB/RIF was calculated to be 72% (64.8%–78.36%). The sensitivity, specificity, Cohen's kappa, p value and diagnostic accuracy of the Xpert MTB/RIF assay for types of EPTB with sample size more than 10, and MGIT as a reference standard are given in Table III and those for various specimens in Table IV.

Forty-seven (26%) patients had drug-resistant TB (DR-TB), of which 15 (8%) were rifampicin-resistant (RR), 2 (1%) had polyresistant, 14 (8%) had MDR, 15 (8%) had pre-extremely DR (XDR) and 1 (1%) had XDR-TB. Of the 14 patients with MDR-TB, Xpert MTB/RIF assay only detected 10 (71%) as RR (p=0.06). Of the 15 pre-XDR cases, Xpert MTB/RIF detected only 8 (53%) patients as RR (p=0.02). The diagnostic accuracy of Xpert MTB/RIF assay compared to MGIT as a reference standard for detection of MDR is 71.43% (45.35%–88.28%) and for pre-XDR is 60% (35.75%–80.18%) in children with EPTB.

DISCUSSION

India has 24% of the total burden of TB in the world. Early detection of TB cases is the key to successful treatment and reduction of disease transmission.[7] Xpert MTB/RIF is a type of nucleic acid amplification technique which uses hemi-nested RT-PCR for amplification of gene sequence specific to *M. tuberculosis* on the *rpoB* gene, using three specific primers. RR-determining region (RRDR) of the *rpoB* gene is probed with molecular beacons for evaluating the RR of the specimen.[8] It is based on hybridization or inhibition of five molecular beacon probes complementary to the wild-type sequence of *rpoB* gene (codons 507 to 533, responsible for 95% of drug resistance mutations in RRDR).[9] There are many advantages of using Xpert MTB/RIF for the diagnosis of TB. The process is almost fully automated, including automation of bacterial lysis, nucleic acid extraction and amplification and amplicon detection; this results in lesser requirement of trained personnel and quicker process than conventional methods.[8] In addition, its high sensitivity and specificity enable diagnosis in smear-negative and often culture-negative TB. The rapidity and robustness of diagnosis help reduce the rate of transmission and allow the early institution of treatment and improved chances for cure. The utility of Xpert assay in the diagnosis of paucibacillary TB is the most important contribution of the test. The Xpert assay has brought about a major change in the speed, simplicity and accuracy of not only diagnosis of TB but also drug resistance to rifampicin in TB, which is accepted as a surrogate for MDR-TB.[9] In contrast, using cultures for diagnosis is time-consuming

and requires laboratories and trained personnel and adherence to biosafety measures.[8]

The Xpert MTB/RIF assay has a high sensitivity and specificity, 95.7% and 99.3%, respectively, for detecting PTB using pulmonary samples.[7] In an Italian study, the Xpert assay showed a sensitivity of 83.1% for EPTB in adults, which is consistent with the results of seven other published studies.[10] Tortoli *et al*. calculated the performance of the Xpert assay for EPTB in adults as well as children. This is the only other study that specifically targeted the performance of the assay in children. It showed a sensitivity of 86.9% and specificity of 99.7% for paediatric samples; the sensitivity and specificity of 81.3% and 99.8%, respectively, were calculated for adult specimens. Overall, the study concluded a sensitivity and specificity of 79% and 97.3%, respectively.[11] Our study concluded Xpert MTB/RIF's sensitivity and specificity of 72% and 72.04%, respectively, for paediatric specimens. Tortoli *et al*. also concluded that the sensitivity for samples of biopsies, urines, pus and CSF exceeded 85%, while it was slightly under 80% for gastric aspirates. In our study, the sensitivity of gastric aspirate was the highest among all types of samples. The limitations of the study by Tortoli *et al*. included failure to report the data of the absolute number of children included and sample tests along with the number of samples tested per child.[11] The variation of results of these studies may be due to differences between patient populations, selection of participants, type of EPTB, the quality and processing of samples and diagnostic gold standard used.[10]

The Xpert assay also comes with its own set of limitations. Rifampicin resistance is taken as a surrogate marker for MDR-TB, but that is not true for all cases. Some strains are only resistant to rifampicin, while some strains are sensitive to rifampicin but resistant to isoniazid,[12,13] which is not detected by this assay. Such cases may not warrant full-line MDR therapy and may also take a longer time to treat. Xpert MTB/RIF also cannot differentiate between the degrees of resistance and between MDR-TB and XDR-TB. As a result, the current gold standard for drug resistance diagnosis is with DST, which detected all clinically relevant resistances. Our study also showed that Xpert MTB/RIF may miss detecting MDR-TB and pre-XDR-TB by finding the sample to be rifampicin-sensitive but which may, in fact, be RR on the conventional DST. A measure that can be taken to increase the detection rates of DR-TB and initiating appropriate treatment earlier is to consider the Xpert assay and conventional microscopy and culture simultaneously in clinically suspected patients. A limitation of our study is the small sample size for each type of specimen.

Conclusion

The Xpert MTB/RIF assay is a useful contribution to the diagnosis of EPTB as it has a high sensitivity and specificity and provides fast results. It also shows good concordance with MGIT results. In resource-limited settings and less accessible areas where it is difficult to establish a sophisticated laboratory for culture and DST conforming to the prescribed biosafety levels, Xpert MTB/RIF provides a viable option. However, MGIT culture and conventional DST are still required to detect DR-TB.

Conflicts of interest. None declared

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